



Figure S2. The structure of the PDE10A2 D674A mutant catalytic domain in complex with cGMP (PDB ID: 2OUU, chain A; wheat) was structurally aligned with the PDE5/6cd (PDB ID: 3JWR, chain A; *green*) bound with P γ_{70-87} (3JWR, chain C; *magenta*) using PyMOL 1.2r2. P γ_{70-87} is shown in surface representation and cGMP is shown in space-filling representation. In the superimposed model, P γ_{70-87} does not contact or clash with cGMP.

```

Hs_PDE6C   WEQGDLE R T V L Q Q Q P I P M M D R N K R D E L P K L Q V G F I D F V C T
ancPDE6    FEQGDLE R K L L N Q E P I P M M D R K K K D E L P K M Q V G F I D S V C T
ancPDE5/6/11 FEQGDLE R K K L N Q E P I P M M D R K K K D E L P K M Q V G F I D S I C L
Nv_PDE5/6  FDQGDLE K E K L N A E P I P M M D R R K K N E L P S M Q V G F I D F I C L
Hm_PDE5/6  FEQGDQ E R S K L S A E P I P M M D R G K H R D L P K M Q V G F I D F V C M
          ↑      ↑      ↑      ↑      ↑↑↑↑      ↑

```

Figure S3. Sequence alignment of the M-loop/ α -helix-15 regions of human *Hs_PDE6C* (P51160), ancestral PDE6, ancestral PDE5/6/11, and PDE5/6-like PDEs from *Nematostella vectensis* (*Nv_PDE5/6*, XP_001634744.1) and *Hydra magnipapillata* (*Hm_PDE5/6*, XP_002161774). The sequences of the catalytic domains of the ancestral PDE6 (*ancPDE6*) and ancestral PDE5/6/11 (*ancPDE5/6/11*) enzymes were reconstructed using the ANCESCON software package (Max-Planck Institute's Bioinformatics Toolkit) and the multiple sequence alignment (ClustalW) of the catalytic domains of the following PDEs: *Homo sapiens* PDE1A (P54750), PDE2A (O00410), PDE3A (Q14432), PDE4A (P27815), PDE5A (O76074), PDE6A (P16499), PDE6B (P35913), PDE6C (P51160), PDE7A (Q13946), PDE8A (O60658), PDE9A (O76083), PDE10A (Q9Y233), PDE11 (Q9HCR9); *Gallus gallus* PDE1 (XP_418850.2), *Danio rerio* PDE4 (XP_695374.3); *Petromyzon marinus* PDE6 (ABO28525.1); *Ciona savignyi* PDE6 (Gene ID ENSCSAVG 00000005480); *Ciona intestinalis* PDE5 (Gene ID ENSCING00000003569), PDE6 (SNAP_CIONA 00000036352), PDE10 (XP_002121759.1); *Strongylocentrotus purpuratus* PDE11 (XP_78196); *Caenorhabditis elegans* PDE2 (NP_001022707.1), PDE3 (NP_871943); *Nematostella vectensis* PDE1 (XP_001629364.1), PDE2 (XP_001630707.1), PDE4 (XP_001638196.1), PDE5 (XP_001631585.1), PDE5/6 (XP_001634744.1), PDE10 (XP_001624188.1), PDE11 (XP_0011635720.1); *Hydra magnipapillata* PDE2 (XP_002162064.1), PDE5 (XP_002162868), PDE5/6 (XP_002161774), PDE10 (XP_002165749.1), PDE11 (XP_002165584.1). Arrows correspond to positions of residues of PDE5/6cd contributing to the interaction with the P γ C-terminus and the H-M loop interface (as in Figure S1).